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(54) IL-4-DERIVED PEPTIDES FOR MODULATION OF THE CHRONIC INFLAMMATORY RESPONSE AND TREATMENT OF AUTOIMMUNE DISEASES

VON IL-4 ABGELEITETE PEPTIDE ZUM MODULIEREN DER CHRONISCHEN ENTZÜNDUNGSREAKTION UND BEHANDLUNG VON AUTOIMMUNKRANKHEITEN

PEPTIDES DÉRIVÉS D IL-4 POUR LA MODULATION DE LA RÉPONSE INFLAMMATOIRE CHRONIQUE ET LE TRAITEMENT DE MALADIES AUTO-IMMUNES

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Description

Technical Field

[0001] The present disclosure relates to small peptides derived from a cytokine, interleukin-4 (IL-4), capable of binding to the IL-4 receptors and inhibiting macrophage activation, and thereby preventing the onset of inflammatory response. The disclosure further relates to use of said peptides for the production of a medicament for the treatment of different pathological conditions, wherein IL-4 plays a prominent role.

Background

[0002] Abnormalities associated with inflammation comprise a large, unrelated group of disorders which underlie a variety of human diseases. Examples of disorders associated with inflammation include asthma, chronic inflammation, and autoimmune diseases including rheumatoid arthritis. Chronic inflammation is a pathological condition characterised by concurrent active inflammation, tissue destruction, and attempts at repair. Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that causes the immune system to attack the joints, where it causes inflammation (arthritis) and destruction. It can also damage some organs, such as the lungs and skin. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility. It is diagnosed with blood tests (especially a test called rheumatoid factor) and X-rays.

[0003] The inflammatory reaction observed in autoimmune disease involves both cellular and soluble players. The cause of RA is not known. It involves complex interactions of various cells, cytokines and enzymes. The disease begins when an inciting antigen gains access to the joint, triggering an immune response. The antigenic stimulus activates CD4+ lymphocytes (T-cells). Once CD4+ T-cells become activated, a complex cascade of biological events take place including stimulation of macrophages, B-cells, fibroblasts, chondrocytes and osteoclasts. Activated macrophages secrete cytokines, such as interleukin-1(IL-1), IL-6, IL-8, IL-15 and tumor necrosis factor-α (TNF- α) (Martinez et al., 2008).

[0004] Interleukin-4 (IL-4) is secreted by CD4+ T-cells (Th2 cells). It is a pleiotropic cytokine, acting on various cell types and tissues. Its action on immune cells results in activation and growth of B cells, IgG and IgE production, MHC class II induction, growth and survival of T cells, Th2 differentiation, enhancement of mast cell growth, enhancement of IL-2 and IL-12-induced interferon-γ (INF- γ) secretion in NK cells, downregulation of C5a and C3a in monocytes and Mo-derived dendritic cells and inhibition of macrophage activation (Agnello et al., 2003; Szehedi et al., 2003; Roland, 2003).

[0005] The structure of recombinant human IL-4 has been determined by both NMR and X-ray diffraction methods in several laboratories. It has a classical 4 helix bundle cytokine structure (Muller et al., 1995). IL-4, like other cytokines, exerts its biological activity by binding to the receptors on the cell surface. One receptor complex is composed of two components, the IL-4R α chain (IL-4Rα) and the IL-2R γ chain (γc, shared by the cytokines IL-2, IL-7, IL-9, IL-15 and IL-21), denoted type I IL-4R, whereas the other receptor complex is composed of IL-4Rα and the IL13 α chain (IL-13Rα1), called type II IL-4R. As γ c is expressed on most hematopoietic and immune cells, IL-4 is assumed to act on these cells through type I IL-4R. In contrast, expression of IL-13Rα1 is limited to some lineages such as B cells in hematopoietic and immune cells, but ubiquitously detected on non-immune cells (Izuhara et al., 2002). Thus IL4 acts on non-immune cells through type II IL-4R/IL-13R.

[0006] Binding IL-4 to its receptor a chain (IL-4Ra) is a crucial event for the generation of a Th2- dominated early immune response. The crystal structure of the intermediate complex between human IL-4 and IL4-BP was determined at 2.3 Å Resolution (PDB ID: 1IAR). It reveals a novel spatial orientation of the two proteins, a small but unexpected conformational change in the receptor-bound IL-4, and an interface with three separate clusters of *trans*-interacting residues (Hage et al., 1999). Crystal structure of the ||4-||4r-common gamma ternary complex has recently been solved (PDB ID: 3BPL; LaPorte et al., 2008).

[0007] Recombinant IL-4 has been through several clinical trials. IL-4 has been shown to be beneficial in patients with psoriasis, effectively correcting imbalances in immune functions (Martin 2003). The safety and tolerability of *Escherichia coli*-derived recombinant human interleukin-4 (rhuIL-4) have been evaluated in phase I and phase II studies in human patients with a variety of malignancies. Clinical trials have demonstrated that subcutaneous administration of rhuIL-4 is safe and well tolerated at doses as high as 5 μg/kg/day and as high as 10 μg/kg when administered 3 times/week. Although preclinical safety studies in cynomolgus monkeys demonstrated a number of adverse effects following repeated daily dosing with rhuIL-4, similar effects have generally not been observed in human patients (Leach et al., 1997). The most common toxicities were elevated liver function tests, nausea/vomiting/diarrhea, malaise/fatigue, edema, headache, myalgias/arthralgias, and fever/chills. Despite promising preclinical growth inhibitory and immunomodulatory effects, IL-4 in this dose and schedule showed only low antitumor activity (Whitehead et al., 1998).

[0008] Many human autoimmune and inflammatory diseases are still treated by a combination of corticosteroids and general immunosuppression. A better understanding of the pathogenesis of these diseases has led to therapies that are more specific. Among these, the recombinant humanized proteins are considered as the future therapies. However,

drugs based on recombinant proteins have several disadvantages including high production cost, big batch-to-batch variation and denaturation during storage.

Summary

[0009] The present invention concerns a fragment of IL-4 that can be chemically synthesized and used as a functional mimetic of IL-4.

[0010] The present invention relates to a compound comprising one or more peptides, each peptide consisting of AQFHRHKQLIRFLKRA (SEQ ID NO:1), or a variant thereof having a different amino acid at one position. A compound comprising such amino acid sequence is according to the invention capable of inhibiting macrophage activation. The present invention also relates to an isolated peptide consisting of AQFHRHKQLIRFLKRA (SEQ ID NO:1), or a variant thereof having a different amino acid at one position.

[0011] Accordingly, another aspect of the invention relates to use of compounds of the invention as medicaments and for the preparation of medicaments and for the use for treatment of a condition or disease wherein inhibiting macrophage activation is part of said treatment. A compound or peptide as defined herein is also provided for use in the treatment of an inflammatory diseases or condition and an autoimmune disease or condition, including rheumatoid arthritis, ischaemic heart disease, inflammatory diseases of the central nervous system, Alzheimer's disease, Parkinson's disease, Huntington's disease, Multiple sclerosis, meningitis and encephalitis.

[0012] The invention further relates to pharmaceutical compositions comprising a peptide of the invention.

Description of Drawings

[0013]

Figure 1. Structure of IL-4 in complex with the ectodomain of IL-4Rα (PDB ID: 1IAR). Location of peptide 1 (SEQ ID NO:2) (left) and peptide3 (SEQ ID NO:3) (right) is indicated in grey.

Figure 2. Structure of IL-4 in complex with the ectodomain of IL-4Rα (PDB ID: 1IAR). Location of peptide3a (SEQ ID NO:1) (left) and peptide4 (SEQ ID NO: 4) (right) is indicated in grey.

Figure 3. Structure of IL-4 in complex with the ectodomain of γc common receptor (PDB ID: 3BPL). Location of peptide 1 (SEQ ID NO:2) (left) and peptide3 (SEQ ID NO:3) (right) is indicated in grey.

Figure 4. Structure of IL-4 in complex with the ectodomain of IL-4Rα and γc common receptor (PDB ID: 3BPL). Location of peptide3a (SEQ ID NO:1) (left) and peptide4 (SEQ ID NO: 4) (right) is indicated in grey.

Figure 5. Effect of IL-4-derived peptide Ph1 (SEQ ID NO:2) on neurite outgrowth in cultures of cerebellar granule neurons. The P2d peptide was used a positive control (see Soroka et al., 2002).

Figure 6. Effect of Ph2 (SEQ ID NO:3) on neurite outgrowth in cultures of cerebellar granule neurons. Level of significance compared to control is represented as followed: *** = $p < 0.001$. Seven independent experiments were performed.

Figure 7. Macrophage secretion of TNF-α when pre-treated with Ph2 (SEQ ID NO:3).

A: Column diagram of the amount of TNF-α released from macrophages when not pre-treated with Ph2 or activated by IFN-γ (striped column), when activated with 0.01 μg/ml IFN-γ (white column) or when pre-treated with 100 μM hydrocortisone and activated with 0.01 μg/ml IFN-γ (black column). Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (white column) are represented as followed:*** = $p < 0.001$. B: Column diagram of the amount of TNF-α released from macrophages when pre-treated with Ph2 in various concentrations before activation with 0.01 μg/ml IFN-γ. Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (0 column) is represented as followed: *** = $p < 0.001$. Results in both figures are shown as percentages of the untreated control, only activated by IFN-γ. Results from six independent experiments are shown for the controls and the Ph2 concentrations 9, 27, 81 and 243 μg/ml.

Figure 8. Binding of Ph2 (SEQ ID NO:3) to IL4rα.
Binding study by applying Surface Plasmon Resonance. A: As a control, binding between IL4 and IL4rα was inves-

tigated by immobilizing IL4rα on a chip and then IL4 was run over the chip in solution. B: Binding between Ph2 and IL4rα was studied by immobilizing Ph2 on the chip and IL4rα was run over the chip in solution. Results were analysed and KD was calculated with the computer software BIAevaluation.

Figure 9. Effect of Ph3 (SEQ ID NO:1) on neurite outgrowth in cultures of cerebellar granule neurons. Level of significance compared to control is represented as followed: ** = p< 0.01. Seven independent experiments were performed.

Figure 10. Macrophage secretion of TNF-α when pre-treated with Ph3 (SEQ ID NO:1). A: Column diagram of the amount of TNF-α released from macrophages when not pre-treated with Ph3 or activated by IFN-γ (striped column), when activated with 0.01 μg/ml IFN-γ (white column) or when pre-treated with 100 μM hydrocortisone and activated with 0.01 μg/ml IFN-γ (black column). Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (white column) are represented as followed: *** = p < 0.001. B: Column diagram of the amount of TNF-α released from macrophages when pre-treated with Ph3 in various concentrations before activation with 0.01 μg/ml IFN-γ. Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (0 column) is represented as followed: *** = p < 0.001. Results in both figures are shown as percentages of the untreated control, only activated by IFN-γ. Results from six independent experiments are shown for the controls and the Ph3 concentrations 9, 27, and 81 μg/ml.

Figure 11. Binding of Ph3 (SEQ ID NO:1) to IL4rα.
Binding study by applying Surface Plasmon Resonance. A: As a control, binding between IL4 and IL4rα was investigated by immobilizing IL4rα on a chip and then IL4 was run over the chip in solution. B: Binding between Ph3 and IL4rα was studied by immobilizing Ph3 on the chip and IL4rα was run over the chip in solution. Results were analysed and KD was calculated with the computer software BIAevaluation.

Figure 12. Effect of Ph4 (SEQ ID NO:4) on neurite outgrowth in cultures of cerebellar granule neurons. Level of significance compared to control is represented as followed: *= p<0.05, ** = p< 0.01. Five independent experiments were performed.

Figure 13. Macrophage secretion of TNF-α when pre-treated with Ph5 (SEQ ID NO:5). A: Column diagram of the amount of TNF-α released from macrophages when not pre-treated with Ph4 or activated by IFN-γ (stripes), when activated with 0.01 μg/ml IFN-γ (white) and when pre-treated with 100 μM hydrocortisone and activated with 0.01 μg/ml IFN-γ (black). B: Column diagram of the amount of TNF-α released from macrophages when pre-treated with 9 μg/ml Ph5 before activation with 0.01 μg/ml IFN-γ. Two independent experiments were performed.

Figure 14. Macrophage secretion of TNF-α when pre-treated with Ph6 (SEQ ID NO:6). A: Column diagram of the amount of TNF-α released from macrophages when not pre-treated with Ph4 or activated by IFN-γ (stripes), when activated with 0.01 μg/ml IFN-γ (white) and when pre-treated with 100 μM hydrocortisone and activated with 0.01 μg/ml IFN-γ (black). B: Column diagram of the amount of TNF-α released from macrophages when pre-treated with various concentrations of Ph6 before activation with 0.01 μg/ml IFN-γ. Two independent experiments were performed.

Figure 15. Macrophage secretion of TNF-α when pre-treated with Ph8 (SEQ ID NO:1). A: Column diagram of the amount of TNF-α released from macrophages when not pre-treated with Ph3 or activated by IFN-γ (striped column), when activated with 0.01 μg/ml IFN-γ (white column) or when pre-treated with 100 μM hydrocortisone and activated with 0.01 μg/ml IFN-γ (black column). Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (white column) are represented as followed: *** = p < 0.001. B: Column diagram of the amount of TNF-α released from macrophages when pre-treated with Ph8 in various concentrations before activation with 0.01 μg/ml IFN-γ. Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (0 column) is represented as followed: *** = p < 0.001. Results in both figures are shown as percentages of the untreated control, only activated by IFN-γ. Results from six independent experiments are shown for the controls and the Ph8 concentrations 9, 27, 81 and 243 μg/ml.

Figure 16. Macrophage secretion of TNF-α when pre-treated with Ph10 (SEQ ID:1).

A: Column diagram of the amount of TNF-α released from macrophages when not pre-treated with Ph10 or activated by IFN-γ (striped column), when activated with 0.01 μg/ml IFN-γ (white column) or when pre-treated with 100 μM hydrocortisone and activated with 0.01 μg/ml IFN-γ (black column). Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (white column) are represented as

followed: **= p < 0.01. B: Column diagram of the amount of TNF-α released from macrophages when pre-treated with Ph10 in various concentrations before activation with 0.01 μg/ml IFN-γ. Level of significance compared to TNF-α amount released from non-pre-treated, activated macrophages (0 column) is represented as followed: **= p < 0.01. Results in both figures are shown as percentages of the untreated control, only activated by IFN-γ. Results from four independent experiments are shown for the controls and the Ph10 concentrations 9, 27, 81 and 243 μg/ml. Only two experiments were performed with the concentration 54 μg/ml Ph10 which does that these data were not included in the statistical analysis.

Figure 17. Macrophage secretion of TNF-α when pre-treated with Ph12 (SEQ ID:19).

A: Column diagram of the amount of TNF-α released from macrophages when not pre-treated with Ph12 or activated by IFN-γ (stripes), when activated with 0.01 μg/ml IFN-γ (white) and when pre-treated with 100 μM hydrocortisone and activated with 0.01 μg/ml IFN-γ (black). B: Column diagram of the amount of TNF-α released from macrophages when pre-treated with Ph12 in various concentrations before activation with 0.01 μg/ml IFN-γ. Two independent experiments were performed.

Detailed description

[0014] The present invention is as defined in the claims.

[0015] A compound according to the present disclosure can be a fragment derived from interleukin-4, or it may be derived from a variant of interleukin-4, such as a natural or recombinant interleukin-4 variant, for example a interleukin-4 variant produced by alternative splicing, or genetic polymorphism, or any type of recombinant interleukin-4.

[0016] A peptide according to the present disclosure is a peptide which is capable of interacting with the IL-4 receptor, modulating IL-4 receptor signalling, activating B-cells, activating growth and survival of T-cells, downregulating C5a and C3a in monocytes and dendritic cells or inhibiting macrophage activation.

[0017] By the terms "modulation" or "modulating" are meant a change, such as an inhibition or stimulation. By the term "interacting" is meant an action, such as binding, between the peptide and the IL-4 receptor which cause an effect.

Amino acid sequence

[0018] Compounds according to the present disclosure comprise a peptide consisting of a contiguous amino acid sequence derived from IL-4 or a fragment or variant thereof.

[0019] In one embodiment the compound according to the present disclosure may comprise a peptide consisting of at most 35 contiguous amino acids which is derived from interleukin-4 (SEQ ID:38) or a fragment thereof, or a variant being at least 70% identical to SEQ ID NO:38 or a fragment thereof.

[0020] The amino acid sequence of the human IL-4 precursor (Swiss-Prot ID: P05112) is:

MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS

(SEQ ID NO:38)

[0021] A peptide sequence according to the present disclosure consists of at most 35 contiguous amino acid residues, such as from 3 to 35 amino acid residues, such as from 3 to 30, for example from 3 to 25, such as from 5 to 25, such as form 7 to 25, such as from 8 to 25, for example from 10 to 25, or from 12 to 25, such as from 14 to 25. Sequences comprising from 5 to 25 contiguous amino acid residues are preferred.

[0022] In one embodiment said peptides of the disclosure comprise at most 35 contiguous amino acids which are derived from an alpha-helix of IL-4.

[0023] By the term "alpha-helix" is meant the common motif in the secondary structure of proteins, the alpha helix (α-helix) is a right- or left-handed coiled conformation, in which every backbone N-H group donates a hydrogen bond to the backbone C=O group of the amino acid four residues earlier.

[0024] In a preferred embodiment said peptides of the present disclosure comprise a sequence with the formula X1-X2-X3, wherein
X1 is L,
X2 is I, Q, G, T, or a charged amino acid; and

X3 is Q, T, or a charged amino acid.

[0025] In one embodiment of the present disclosure X2 is I or Q.

[0026] In one embodiment of the present disclosure X2 is I.

[0027] In one embodiment of the present disclosure X2 is Q.

[0028] In one embodiment of the present disclosure X2 is a charged amino acid.

[0029] In one embodiment of the present disclosure X3 is a charged amino acid.

[0030] In one embodiment of the present disclosure X3 is R or E.

[0031] In one embodiment of the present disclosure X3 is R.

[0032] In one embodiment of the present disclosure X3 is E.

[0033] In one embodiment of the present disclosure X3 is Q or T.

[0034] In one embodiment of the present disclosure X1 is L, X2 is I, and X3 is R.

[0035] In one embodiment of the present disclosure X1 is L, X2 is Q and X3 is E.

[0036] In a preferred embodiment of the present disclosure said peptides consist of an amino acid sequence selected from one of the following amino acid sequences:

AQFHRHKQLIRFLKRA	SEQ ID NO:1
AITLQEIIKTLNSA	SEQ ID NO:2
ARFLKRLDRNLWGG	SEQ ID NO:3
AERLKTIMREKYSKS	SEQ ID NO:4
LQEIKTLN	SEQ ID NO:5
KRLQQNLFGG	SEQ ID NO:6
Ac-AQFHRHKQLIRFLKRA	SEQ ID NO:7
QEIIKKL	SEQ ID NO:8
AIQNQEEIKYLNS	SEQ ID NO:9
AIILQEI	SEQ ID NO:10
IVLQEII	SEQ IQ NO:11
TLGEIIKGVNS	SEQ ID NO:12
VTLIDHSEEIFKTLN	SEQ ID NO:13
LQERIKSLN	SEQ ID NO:14
RLDRENVAVYNLW	SEQ ID NO:15
LRSLDRNL	SEQ ID NO:16
RLLRLDRN	SEQ ID NO:17
RFLKRYFYNLEENL	SEQ ID NO:18
RNKQVIDSLAKFLKR	SEQ ID NO:19
RHKALIR	SEQ ID NO:20
KKLI RYLK	SEQ ID NO:21
RHKTLIR	SEQ ID NO:22
MQDKYSKS	SEQ ID NO:23
AERVKIEQREYKKYS	SEQ ID NO:24
SQLIRFLKRLA	SEQ ID NO:25
TVTDIFAASKNTT	SEQ ID NO:26
TLENFLERLKTA	SEQ ID NO:27
TEKEVLRQFYSA	SEQ ID NO:28
KTLTELTKTLNS	SEQ ID NO:29
AHKEIIKTLNSLQKA	SEQ ID NO:30
AKTLSTELTVTA	SEQ ID NO:31
STLENFLERLA	SEQ ID NO:32
NEERLKTIMRA	SEQ ID NO:33
RAATVLRQFYSR	SEQ ID NO:34
KTLNSLTEQKT	SEQ ID NO:35
AHRHKQLIRA	SEQ ID NO:36
ATAQQFHRHKQA	SEQ ID NO:37,

or a variant or fragment thereof.

[0037] In one embodiment the said peptides of the disclosure consist of an amino acid sequence selected from one of the following amino acid sequences:

AQFHRHKQLIRFLKRA	(SEQ ID NO:1)
Ac-AQFHRHKQLIRFLKRA	(SEQ ID NO:7)
RHKALIR	(SEQ ID NO:20)
KKLI RYLK	(SEQ ID NO:21)
RHKTLIR	(SEQ ID NO:22)
SQLIRFLKRLA	(SEQ ID NO:25)
AHRHKQLIRA	(SEQ ID NO:36),

or a variant or fragment thereof.

[0038] In one embodiment the said peptides of the disclosure consist of an amino acid sequence selected from one of the following amino acid sequences:

AITLQEIIKTLNSA	(SEQ ID NO:2)
LQEIKTLN	(SEQ ID NO:5)
AIILQEI	(SEQ ID NO:10)
IVLQEII	(SEQ ID NO:11)
LQERIKSLN	(SEQ ID NO:14)
AHKEIIKTLNSLQKA	(SEQ ID NO:30),

or a variant or fragment thereof.

[0039] In the present context the standard one-letter code for amino acid residues as well as the standard three-letter code are applied. Abbreviations for amino acids are in accordance with the recommendations in the IUPAC-IUB Joint Commission on Biochemical Nomenclature Eur. J. Biochem, 1984, vol. 184, pp 9-37. Throughout the description and claims either the three letter code or the one letter code for natural amino acids are used. Where the L or D form has not been specified it is to be understood that the amino acid in question has the natural L form, cf. Pure & Appl. Chem. Vol. (56(5) pp 595-624 (1984) or the D form, so that the peptides formed may be constituted of amino acids of L form, D form, or a sequence of mixed L forms and D forms.

[0040] Where nothing is specified it is to be understood that the C-terminal amino acid of a peptide for use according to the disclosure exists as the free carboxylic acid, this may also be specified as "-OH". However, the C-terminal amino acid of a peptide for use according to the disclosure may be the amidated derivative, which is indicated as "-NH_2". Where nothing else is stated the N-terminal amino acid of a polypeptide comprises a free amino-group, this may also be specified as "H-".

[0041] A peptide, fragment or variant thereof according to the disclosure can also comprise one or several unnatural amino acids.

[0042] A preferred peptide according to the disclosure is an isolated contiguous peptide sequence which comprises at most 35 amino acid residues of IL-4. It is understood that all peptides according to the disclosure comprise at least one amino acid sequence selected from any of the sequences SEQ ID NOs: 1-37 or a fragment or variant thereof.

[0043] Thus, some embodiments of the present disclosure may relate to a peptide comprising a fragment of a sequence selected from SEQ ID NOs:1 to 37. Another embodiment may relate to variants of SEQ ID NOs:1-37.

[0044] In one embodiment of the present disclosure a variant fragment varies compared to a fragment of SEQ ID NO 38. A variant fragment may differ from a fragment of SEQ ID NO 38 by having a different amino acid at one or more positions. Preferably the variant differs from the fragment of SEQ ID NO 38 at up to 10 amino acid positions, more preferably at up to 8 position, such as up to 6 positions, for example up to 5 positions, such as at 4, 3, 2 or 1 position. Such variants may also differ from a fragment of SEQ ID NO 38 in other ways, such as by having one or more chemical modifications.

[0045] A variant according to the disclosure of an amino acid sequence selected from the sequences SEQ ID NOs: 1-38 may be

i) an amino acid sequence which has at least 70% identity with a selected sequence, such as 71-75% identity, for example 76-80% identity, such as 81-85% identity, such as 86-90% identity, for example 91-95% identity, such as 96-99% identity, wherein the identity is defined as a percentage of identical amino acids in said sequence when it is collated with the selected sequence. The identity between amino acid sequences may be calculated using well

known algorithms such as BLOSUM 30, BLOSUM 40, BLOSUM 45, BLOSUM 50, BLOSUM 55, BLOSUM 60, BLOSUM 62, BLOSUM 65, BLOSUM 70, BLOSUM 75, BLOSUM 80, BLOSUM 85, or BLOSUM 90;

ii) an amino acid sequence which has at least 70% positive amino acid matches with a selected sequence, such as 71-80% positive amino acid matches, for example 81-85% positive amino acid matches, such as 86-90% positive amino acid matches, for example 91-95% positive amino acid matches, such as 96-99% positive amino acid matches, wherein the positive amino acid match is defined as the presence at the same position in two compared sequences of amino acid residues which has similar physical and/or chemical properties. Preferred positive amino acid matches of the present disclosure are K to R, E to D, L to M, Q to E, I to V, I to L, A to S, Y to W, K to Q, S to T, N to S and Q to R;

iii) an amino acid sequence which is identical to a selected sequence, or it has at least 70% identity with said sequence such as 71-80% identity, for example 81-85% identity, such as 86-90% identity, for example 91-95% identity, such as 96-99% identity, or has at least 75% positive amino acid matches with the selected sequence, such as 76-80% positive amino acid matches, for example 81-85% positive amino acid matches, such as 86-90% positive amino acid matches, for example 91-95% positive amino acid matches, such as 96-99 % positive amino acid matches, and comprises other chemical moieties, e. g. phosphoryl, sulphur, acetyl, glycosyl moieties.

[0046] The term "variant of a peptide sequence" also means that the peptide sequence may be modified, for example by substitution of one or more of the amino acid residues. Both L-amino acids and D-amino acids may be used. Other modification may comprise derivatives such as esters, sugars, etc., for example methyl and acetyl esters, as well as polyethylene glycol modifications.

[0047] Furthermore, an amine group of the peptide may be converted to amides, wherein the acid part of the amide is a fatty acid.

[0048] According to the present disclosure, variants of the amino acid sequences may comprise, within the same variant, or fragments thereof or among different variants, or fragments thereof, at least one substitution, such as a plurality of substitutions introduced independently of one another. Variants of the complex, or fragments thereof may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one alanine (Ala) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one valine (Val) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one leucine (Leu) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, variants, or fragments thereof, wherein at least one isoleucine (Ile) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, variants, or fragments thereof wherein at least one aspartic acids (Asp) of said variant, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Glu, Asn, and Gln, and independently thereof, variants, or fragments thereof, wherein at least one aspargine (Asn) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, variants, or fragments thereof, wherein at least one glutamine (Gln) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and wherein at least one phenylalanine (Phe) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, variants, or fragments thereof, wherein at least one tyrosine (Tyr) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, variants, or fragments thereof, wherein at least one arginine (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, variants, or fragments thereof, wherein at least one lysine (Lys) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, variants, or fragments thereof, and independently thereof, variants, or fragments thereof, and wherein at least one proline (Pro) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, variants, or fragments thereof, wherein at least one cysteine (Cys) of said variants, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

[0049] It thus follows from the above that the same variant of a peptide fragment, or fragment of said variant may comprise more than one conservative amino acid substitution from more than one group of conservative amino acids

as defined herein above. The term "conservative amino acid substitution" is used synonymously herein with the term "homologous amino acid substitution".

[0050] The groups of conservative amino acids are as the following:

A, G (neutral, weakly hydrophobic),
Q, N, S, T (hydrophilic, non-charged)
E, D (hydrophilic, acidic)
H, K, R (hydrophilic, basic)
L, P, I, V, M, F, Y, W (hydrophobic, aromatic)
C (cross-link forming)

[0051] Conservative substitutions may be introduced in any position of a preferred predetermined peptide for use according to the disclosure or fragment thereof. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a non-conservative substitution in any one or more positions.

[0052] A non-conservative substitution leading to the formation of a variant fragment of the peptide for use according to the disclosure would for example differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on peptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

[0053] Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like.

[0054] A peptide according to the disclosure is a peptide which is capable of interacting with the IL-4 receptor.

[0055] In one embodiment the peptide according to the disclosure is capable of modulating IL-4 receptor signalling.

[0056] In a preferred embodiment the peptide according to the disclosure is capable of stimulating IL-4 signalling. In another preferred embodiment the peptide according to the disclosure is capable of inhibiting IL-4 receptor signalling.

[0057] In another embodiment the peptide according to the disclosure is capable of activating B-cells.

[0058] In a further embodiment the peptide according to the disclosure is capable of activating growth and survival of T-cells.

[0059] In another embodiment the peptide according to the disclosure is capable of downregulating C5a and C3a in monocytes and dendritic cells.

[0060] In yet another embodiment the peptide according to the disclosure is capable of inhibiting macrophage activation.

[0061] Both fragments and variants of amino acid sequences according to the disclosure are functional equivalents of said sequences.

[0062] By the term "functional equivalent" of an amino acid sequence is in the present context meant a molecule which meets the criteria for a variant or a fragment of said amino acid sequence described above and which is capable of one or more functional activities of said sequence or a compound comprising said sequence. In a preferred embodiment, the functional equivalent of an amino acid sequence according to the disclosure, is capable of interacting with the IL-4 receptor and modulate IL-4 receptor signalling.

[0063] The disclosure relates both to isolated peptides according to the disclosure and fusion proteins comprising peptides according to the disclosure.

[0064] In one embodiment, the peptide according to the disclosure is an isolated peptide. By the term "isolated peptide" is meant that the peptide according to the disclosure is an individual compound and not a part of another compound. The isolated peptide may be produced by use of any recombinant technology methods or chemical synthesis and separated from other compounds, or it may be separated from a longer polypeptide or protein by a method of enzymatic or chemical cleavage and further separated from other protein fragments.

[0065] The peptide sequence may be present in the compound as a single copy, i.e. formulated as a monomer of the peptide sequence, or it may be present as several copies of the same sequence, e.g. as a multimer comprising two or more copies of a sequence selected from SEQ ID NOs:1-37, or two or more copies of a fragment or a variant of said sequence.

[0066] An isolated peptide according to the disclosure may comprise a fragment of interleukin-4 which consists of a contiguous amino acid sequence derived from interleukin-4, selected from SEQ ID NOs:1-37 or a variant thereof. According to the present disclosure the isolated peptide may consist of one or more of the sequences SEQ ID NOs:1-37.

Production of peptide sequences

[0067] The peptide sequences of the present disclosure may be prepared by any conventional synthetic methods, recombinant DNA technologies, enzymatic cleavage of full-length proteins which the peptide sequences are derived from, or a combination of said methods.

Synthetic preparation

[0068] The methods for synthetic production of peptides are well known in the art. Detailed descriptions as well as practical advice for producing synthetic peptides may be found in Synthetic Peptides: A User's Guide (Advances in Molecular Biology), Grant G. A. ed., Oxford University Press, 2002, or in: Pharmaceutical Formulation: Development of Peptides and Proteins, Frokjaer and Hovgaard eds., Taylor and Francis, 1999.

[0069] Peptides may for example be synthesised by using Fmoc chemistry and with Acm-protected cysteines. After purification by reversed phase HPLC, peptides may be further processed to obtain for example cyclic or C- or N-terminal modified isoforms. The methods for cyclization and terminal modification are well-known in the art and described in detail in the above-cited manuals.

[0070] In a preferred embodiment the peptide sequences are produced synthetically, in particular, by the Sequence Assisted Peptide Synthesis (SAPS) method.

[0071] Peptides may be synthesised either batch-wise in a polyethylene vessel equipped with a polypropylene filter for filtration or in the continuous-flow version of the polyamide solid-phase method (Dryland, A. and Sheppard, R.C., (1986) J.Chem. Soc. Perkin Trans. I, 125 - 137) on a fully automated peptide synthesiser using 9-fluorenylmethyloxy-carbonyl (Fmoc) or tert. -Butyloxycarbonyl, (Boc) as N-a-amino protecting group and suitable common protection groups for side-chain functionality's.

Recombinant preparation

[0072] Thus, in one embodiment the peptides are produced by use of recombinant DNA technologies.

[0073] The DNA sequence encoding a peptide or the corresponding full-length protein the peptide originates from may be prepared synthetically by established standard methods, e.g. the phosphoamidine method described by Beaucage and Caruthers, 1981, Tetrahedron Lett. 22:1859-1869, or the method described by Matthes et al., 1984, EMBO J. 3:801-805. According to the phosphoamidine method, oligonucleotides are synthesised, e.g. in an automatic DNA synthesiser, purified, annealed, ligated and cloned in suitable vectors.

[0074] The DNA sequence encoding a peptide may also be prepared by fragmentation of the DNA sequences encoding the corresponding full-length protein of peptide origin, using DNAase I according to a standard protocol (Sambrook et al., Molecular cloning: A Laboratory manual. 2 rd ed., CSHL Press, Cold Spring Harbor, NY, 1989). The present disclosure relates to full-length proteins selected from the groups of proteins identified above. The DNA encoding the full-length proteins of the disclosure may alternatively be fragmented using specific restriction endonucleases. The fragments of DNA are further purified using standard procedures described in Sambrook et al., Molecular cloning: A Laboratory manual. 2 rd ed., CSHL Press, Cold Spring Harbor, NY, 1989.

[0075] The DNA sequence encoding a full-length protein may also be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the full-length protein by hybridisation using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989). The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., 1988, Science 239:487-491.

[0076] The DNA sequence is then inserted into a recombinant expression vector, which may be any vector, which may conveniently be subjected to recombinant DNA procedures. The choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

[0077] In the vector, the DNA sequence encoding a peptide or a full-length protein should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the coding DNA sequence in mammalian cells are the SV 40 promoter (Subramani et al., 1981, Mol. Cell Biol. 1:854-864), the MT-1 (metallothionein gene) promoter (Palmiter et al., 1983, Science 222: 809-814) or the adenovirus 2 major late promoter. A suitable promoter for use in insect cells is the polyhedrin promoter (Vasuvedan et al., 1992, FEBS Lett. 311:7-11). Suitable promoters for use in

yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., 1980, J. Biol. Chem. 255:12073-12080; Alber and Kawasaki, 1982, J. Mol. Appl. Gen. 1: 419-434) or alcohol dehydrogenase genes (Young et al., 1982, in Genetic Engineering of Microorganisms for Chemicals, Hollaender et al, eds., Plenum Press, New York), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., 1983, Nature 304:652-654) promoters. Suitable promoters for use in filamentous fungus host cells are, for instance, the ADH3 promoter (McKnight et al., 1985, EMBO J. 4:2093-2099) or the tpiA promoter.

[0078] The coding DNA sequence may also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.) or (for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) promoters. The vector may further comprise elements such as polyadenylation signals (e.g. from SV 40 or the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV 40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

[0079] The recombinant expression vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. An example of such a sequence (when the host cell is a mammalian cell) is the SV 40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or one which confers resistance to a drug, e.g. neomycin, hydromycin or methotrexate.

[0080] The procedures used to ligate the DNA sequences coding the peptides or full-length proteins, the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

[0081] To obtain recombinant peptides the coding DNA sequences may be usefully fused with a second peptide coding sequence and a protease cleavage site coding sequence, giving a DNA construct encoding the fusion protein, wherein the protease cleavage site coding sequence positioned between the HBP fragment and second peptide coding DNA, inserted into a recombinant expression vector, and expressed in recombinant host cells. In one embodiment, said second peptide is selected from, but not limited by the group comprising glutathion-S-reductase, calf thymosin, bacterial thioredoxin or human ubiquitin natural or synthetic variants, or peptides thereof. According to the present disclosure, a peptide sequence comprising a protease cleavage site may be the Factor Xa, with the amino acid sequence *IEGR,* enterokinase, with the amino acid sequence *DDDDK,* thrombin, with the amino acid sequence *LVPR*/*GS,* or *Acharombacter lyticus,* with the amino acid sequence X *K*X, cleavage site.

[0082] The host cell into which the expression vector is introduced may be any cell which is capable of expression of the peptides or full-length proteins, and is preferably a eukaryotic cell, such as invertebrate (insect) cells or vertebrate cells, e.g. *Xenopus laevis* oocytes or mammalian cells, in particular insect and mammalian cells. Examples of suitable mammalian cell lines are the HEK293 (ATCC CRL-1573), COS (ATCC CRL-1650), BHK (ATCC CRL-1632, ATCC CCL-10) or CHO (ATCC CCL-61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159, 1982, pp. 601-621; Southern and Berg, 1982, J. Mol. Appl. Genet. 1:327-341; Loyter et al., 1982, Proc. Natl. Acad. Sci. USA 79: 422-426; Wigler et al., 1978, Cell 14:725; Corsaro and Pearson, 1981, in Somatic Cell Genetics 7, p. 603; Graham and van der Eb, 1973, Virol. 52:456; and Neumann et al., 1982, EMBO J. 1:841-845.

[0083] Alternatively, fungal cells (including yeast cells) may be used as host cells. Examples of suitable yeast cells include cells of *Saccharomyces spp.* or *Schizosaccharomyces spp.,* in particular strains of *Saccharomyces cerevisiae.* Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus spp.* or *Neurospora spp.,* in particular strains of *Aspergillus oryzae* or *Aspergillus niger*. The use of *Aspergillus spp.* for the expression of proteins is described in, e.g., EP 238 023.

[0084] The medium used to culture the cells may be any conventional medium suitable for growing mammalian cells, such as a serum-containing or serum-free medium containing appropriate supplements, or a suitable medium for growing insect, yeast or fungal cells. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection).

[0085] The peptides or full-length proteins recombinantly produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. HPLC, ion exchange chromatography, affinity chromatography, or the like.

Medicament

[0086] It is an objective of the disclosure to provide a compound capable of modulating the activity of IL-4, said compound according to the disclosure can be used as a medicament for the treatment of diseases, wherein modulation of IL-4 signalling may be considered as an essential condition for curing.

[0087] Accordingly, the disclosure relates to the use of one or more of the peptides comprising a sequence derived

from IL-4 or a fragment or variant thereof for the manufacture of a medicament.

[0088] In one embodiment the medicament comprises at least one of the amino acid sequences set forth in SEQ ID NOS: 1-37 or fragments or variants of said sequences. In another embodiment the medicament of the disclosure comprises an antibody capable of binding to an epitope in IL-4 or a fragment thereof or a fragment or variant of said antibody.

[0089] The medicament of the disclosure comprises an effective amount of one or more of the compounds as defined above, or a composition comprising a compound as defined above, in combination with pharmaceutically acceptable additives. Such medicament may suitably be formulated for oral, percutaneous, subcutaneous, topical, intramuscular, intravenous, intracranial, intrathecal, intracerebroventricular, nasal, intranasal or pulmonal administration or parental administration supplemented with intraarticular administration into or near joint capsules.

[0090] Strategies in formulation development of medicaments and compositions based on the peptides of the present disclosure generally correspond to formulation strategies for any other protein-based drug product. Potential problems and the guidance required to overcome these problems are dealt with in several textbooks, e.g. "Therapeutic Peptides and Protein Formulation. Processing and Delivery Systems", Ed. A.K. Banga, Technomic Publishing AG, Basel, 1995.

[0091] Injectables are usually prepared either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. The preparation may also be emulsified. The active ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like, and combinations thereof. In addition, if desired, the preparation may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or which enhance the effectiveness or transportation of the preparation.

[0092] Formulations of the compounds of the disclosure can be prepared by techniques known to the person skilled in the art. The formulations may contain pharmaceutically acceptable carriers and excipients including microspheres, liposomes, microcapsules, nanoparticles or the like.

[0093] The preparation may suitably be administered by injection, optionally at the site, where the active ingredient is to exert its effect. Additional formulations which are suitable for other modes of administration include suppositories, nasal, pulmonal and, in some cases, oral formulations. For suppositories, traditional binders and carriers include polyalkylene glycols or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient(s) in the range of from 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and generally contain 10-95% of the active ingredient(s), preferably 25-70%.

[0094] Other formulations are such suitable for nasal and pulmonal administration, e.g. inhalators and aerosols.

[0095] The active compound may be formulated as neutral or salt forms.

[0096] Pharmaceutically acceptable salts include acid addition salts (for example formed with the free amino groups of the peptide compound) and which are formed with inorganic acids such as, for example, hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, nitric acids and the like, or such organic acids as formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pamoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, EDTA, glycolic, p-aminobenzoic, glutamic, benzenesulfonic, p-toluenesulfonic acids and the like. Salts formed with the free carboxyl group may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0097] Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2. Examples of metal salts include lithium, sodium, potassium, magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium, tetramethylammonium salts and the like.

[0098] The preparations are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated, including, e.g. the weight and age of the subject, the disease to be treated and the stage of disease. Suitable dosage ranges are per kilo body weight normally of the order of several hundred μg active ingredient per administration with a preferred range of from about 0.1 μg to 5000 μg per kilo body weight. Using monomeric forms of the compounds, the suitable dosages are often in the range of from 0.1 μg to 5000 μg per kilo body weight, such as in the range of from about 0.1 μg to 3000 μg per kilo body weight, and especially in the range of from about 0.1 μg to 1000 μg per kilo body weight. Using multimeric forms of the compounds, the suitable dosages are often in the range of from 0.1 μg to 1000 μg per kilo body weight, such as in the range of from about 0.1 μg to 750 μg per kilo body weight, and especially in the range of from about 0.1 μg to 500 μg per kilo body weight such as in the range of from about 0.1 μg to 250 μg per kilo body weight. In particular when administering nasally smaller dosages are used than when administering by other routes. Administration may be performed once or may be followed by subsequent administrations. The dosage will also depend on the route of admin-

istration and will vary with the age and weight of the subject to be treated. A preferred dosage of multimeric forms would be in the interval 1 mg to 70 mg per 70 kg body weight.

[0099] For most indications a localised or substantially localised application is preferred.

[0100] Some of the compounds of the present disclosure are sufficiently active, but for some of the others, the effect will be enhanced if the preparation further comprises pharmaceutically acceptable additives and/or carriers. Such additives and carriers will be known in the art. In some cases, it will be advantageous to include a compound, which promotes delivery of the active substance to its target.

[0101] In many instances, it will be necessary to administrate the formulation multiple times. Administration may be a continuous infusion, such as intraventricular infusion or administration in more doses such as more times a day, daily, more times a week, weekly, etc. It is preferred that administration of the medicament is initiated before or shortly after the individual has been subjected to the factor(s) that may lead to cell death. Preferably the medicament is administered within 8 hours from the factor onset, such as within 5 hours from the factor onset. Many of the compounds exhibit a long term effect whereby administration of the compounds may be conducted with long intervals, such as 1 week or 2 weeks.

[0102] In connection with the use in nerve guides, the administration may be continuous or in small portions based upon controlled release of the active compound(s). Furthermore, precursors may be used to control the rate of release and/or site of release. Other kinds of implants and well as oral administration may similarly be based upon controlled release and/or the use of precursors.

[0103] As discussed above, the present disclosure relates to treatment of individuals for inducing differentiation, modulating proliferation, stimulate regeneration, neuronal plasticity and survival of cells *in vitro* or *in vivo*, the treatment involving administering an effective amount of one or more compounds as defined above.

[0104] Another strategy for administration is to implant or inject cells capable of expressing and secreting the compound in question. Thereby the compound may be produced at the location where it is going to act.

Treatment

[0105] The compounds according to the disclosure are particularly useful for treating inflammatory diseases and conditions. The compounds are useful for the diseases and conditions mentioned below, in particular useful for the treatment of inflammation in association with Rheumatoid arthritis and autoimmune diseases, as well as with Alzheimer's disease, Parkinson's disease and Huntington's disease.

[0106] Examples of disorders associated with inflammation that can be treated with the compounds of the disclosure include; neuroinflammation, Alzheimer's disease, Parkinson's disease and Huntington's disease, asthma and other allergic reactions, autoimmune diseases such as Acute disseminated encephalomyelitis (ADEM), Addison's disease, ALS, Ankylosing spondylitis, Antiphospholipid antibody syndrome (APS), Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Bullous pemphigoid, Coeliac disease, Chagas disease, Chronic obstructive pulmonary disease, Dermatomyositis, Diabetes mellitus type 1, Endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's disease, Hidradenitis suppurativa, Idiopathic thrombocytopenic purpura, Interstitial cystitis, Lupus erythematosus, Morphea, Multiple sclerosis, Myasthenia gravis, Narcolepsy, Neuromyotonia, Pemphigus Vulgaris, Pernicious anaemia, Polymyositis, Primary biliary cirrhosis, Rheumatoid arthritis, Schizophrenia, Scleroderma, Sjögren's syndrome, SLE, Temporal arteritis (also known as "giant cell arteritis"), Vasculitis, Vitiligo, Wegener's granulomatosis; chronic inflammation, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, transplant rejection, vasculitis, osteoarthritis, tendovaginitis, and arthritis.

[0107] The treatment may also be of persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions, inflammatory disease of the central nervous system, such as meningitis, encephalitis, inflammatory and toxic neuropathy, including acute infective polyneuritis, inflammatory disorders with tissue damage, HIV, hepatitis, osteoarthritis, tendovaginitis, and arthritis.

[0108] In one embodiment the treatment may be of non-immune diseases with aetiological origins in inflammatory processes including cancer, atherosclerosis, and ischaemic heart disease.

Examples

Example 1

[0109] Four peptides derived from IL-4 were designed and synthesized (SEQ ID NOs:1-4). Mapping of the location of the peptides was performed employing PyMOL™ software, based on PyMOL v0.99 (DeLano Scientific LLC, South San Francisco, California, U.S.A). This was done based on the crystal structure of the ternary complex of human Il4-Il4r-Il13ra, PDB ID: 3BPN and 3BPL (LaPorte et al., 2008).

[0110] IL-4 interacts with two fibronectin type III modules (FN3-1 and FN3-2) of the extracellular part of the IL-4Rα)

(Figure 1 and 2). IL-4 interacts with two fibronectin type III modules (FN3-1 and FN3-2) of the extracellular part of IL-4Rα and γc (figures 3 and 4).

Example 2

[0111] 4 peptides derived from IL-4 were tested in a neurite outgrowth assay whether they had any biological activity.

[0112] Cerebellar granular neurons (CGN) were prepared from 3 or 7 postnatal (P) day Wistar rats (Charles River, Sulzfeld, Germany or Taconic, Ejby, Denmark). Cerebella were cleared of meninges and blood vessels, roughly homogenized by chopping, and trypsinized with trypsin from Sigma-Aldrich (Brøndby, Denmark). The neurons were washed in the presence of DNAse 1 and soybean trypsin inhibitor (Sigma-Aldrich), and cellular debris was pelleted by centrifugation before plating. For single-cell culture experiments, P7 CGNs were plated at a density of 10,000 cells/well onto uncoated eight-well Lab-Tek chamber slides (NUNC, Slangerup, Denmark) in Neurobasal-A medium supplemented with 0.4% (w/v) BSA. Peptides at various concentrations were added to the medium immediately after plating, and cells were maintained at 37°C and 5% CO_2 for 24 h. Cultures then were fixed, blocked and incubated with polyclonal rabbit antibody against rat GAP-43 (Chemicon, Temecula, CA, USA) followed by incubation with secondary Alexa Fluor488 goat anti-rabbit antibody (Molecular Probes, Eugene, OR, USA) as previously described (Neiiendam et al., 2004). The immunostained cultures were all recorded by computer-assisted fluorescence microscopy using a Nikon Diaphot inverted microscope (Nikon, Japan) equipped with a Nikon Plane 20x objective. Images were captured with a charge-coupled device video camera (Grundig Electronics, Nurnberg, Germany) using the software package Prima developed at the Protein Laboratory (University of Copenhagen, Copenhagen, Denmark). The length of neuronal processes per cell was estimated using the software package Process Length developed at the Protein Laboratory (Ronn *et al.* 2000). For estimation of neurite outgrowth, at least 200 ± 20 cells were processed for each group in each individual experiment.

Results:

[0113] Peptides with the SEQ ID NOs: 1, 2, 3, and 4, from the IL-4 binding site were found to induce a neuritogenic response from primary neurons. The results of the effect of SEQ ID NO:1 2, 3 and 4 on cerebellar neurite outgrowth are shown in figures 5, 6, 9, and 12, respectively.

Example 3

[0114] Primary macrophage cells (or cells of the AMJ2C8 macrophage cell line, see Ryan et al., 1997) can be cultured for 24 h at a density of 6 x 10^{-5} cells/ml in 12-well plates (Nunc, Slangerup, Denmark) at 37°C, in 5% CO_2 and 95% humidity. For determination of TNF-α release in response to LPS stimulation, triplicate cultures were cultured in DMEM with 10% FCS for 24 h and then stimulated with 0-10μg/ml LPS for an additional 24 h period, after which culture supernatants were collected. Determination of TNF-α concentrations in conditioned media from LPS-treated macrophages was done employing the L929 fibroblast-like cells which were sensitive to TNF-α upon exposure to actinomycin D (He et al., 2002). L929 cells were seeded in 96-well plates at a density of 20.000 cells per well and maintained at 37°C, 5% CO_2, RPMI 1640 supplemented with 10% FCS and 0.5% penicillin-streptomycin. At 1 h prior to use as the TNF-α bioassay, L929 cells were pre-treated with 5μg/ml actrinomycin D (Sigma), and further incubated with conditioned medium, in various dilutions, from LPS-treated macrophage cultures. Cell viability was than evaluated using the CellTiter 96 assay (Promega, Madison, WI, USA).

Macrophage activation test-system

[0115]

- Macrophages were seeded in 6 well multidish with 9.6cm^2 per well, in the density 10.000 cells/well.
- Peptides or protein with potential anti-inflammatory effects were added to the culture. As negative control, medium was added to one well and as a positive control, 100 μM hydrocortisone was added to one well.
- Cell cultures were incubated for 24h at 37 °C.
- IFN-γ was added to the macrophage cultures to activate macrophages in the concentration 0.01 μg/ml. As control no IFN-γ but medium was added to one well.
- Fibroblast cells were seeded in a 96 well plate, in the concentration 0.2 x 10^2 cells/ml.
- Both cell cultures were incubated for 24h at 37 °C.

[0116] Conditioned medium from macrophages was collected by spinning the cell solution for 5 min at 1200 rpm. The conditioned medium was added to fibroblasts, TNF-α was added for the titration curve and finally actinomycin D was

added to the fibroblasts in the concentration 0.5 μg/ml.

Results:

[0117] The effect of peptides with SEQ ID NOs:1, 3, 5, 6, and 19 on inhibition of an inflammatory response in macrophage cell cultures was tested.. Results are shown in Figures 7, 10, and 13-17.

Example 4

Binding studies using Surface Plasmon Resonance (SPR) analysis

[0118] Recombinant IL4Rα was immobilized on a CM5 sensor chip. The immobilization process was done by activating the carboxymethylated dextran matrix with 35 μl activation solution followed by an injection of protein in 10mM sodium acetate solution (pH 5.0). After a desired level of protein was immobilized 35 μl of deactivation solution is injected to deactivate any free carboxymethylated groups in the dextran matrix. One flow cell was always empty as a control. Each analyte (recombinant IL-4 or IL-4-derived peptides) was diluted in PBS and injected at a flow rate of 10 μl/min. The obtained data was analyzed by performing a non-linear curve fitting using the software BIAevaluation v.4 from Biacore. The curves were fitted to a 1:1 Langmuir binding model which describes the interaction of two molecules in 1:1 complex. The affinity constant (K_D) was calculated from the association rate constant (k_a) and the dissociation rate constant (k_d). This was done by using the following formula, where L is the immobilized ligand, A the analyte, and LA is the analyte-ligand complex:

Langmuir 1:1 model:

$$L + A \underset{\leftarrow k_d}{\overset{k_a \rightarrow}{}} LA$$

Rate of decreasing ligand concentration:

$$\frac{d[L]}{dt} = -(k_a * [L] * [A] - k_d * [LA])$$

Rate of increasing product concentration:

$$\frac{d[LA]}{dt} = k_a * [L] * [A] - k_d * [LA]$$

$$\text{At steady state: } \frac{d[LA]}{dt} = 0$$

$$\Rightarrow k_a * [L] * [A] - k_d * [LA] = 0$$

$$\Rightarrow \frac{[L] * [A]}{[LA]} = \frac{k_d}{k_a} = K_D$$

Results:

[0119] Binding between Ph2 (SEQ ID NO:3), and IL4rα, and between Ph3 (SEQ ID NO:1) and IL4rα was studied. The results are shown in Figures 8 and 11, respectively.

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SEQUENCE LISTING

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Claims

1. A compound consisting of one or more peptides, each peptide consisting of AQFHRHKQLIRFLKRA (SEQ ID NO:1), or a variant thereof having a different amino acid at one position, wherein said peptide is capable of inhibiting macrophage activation.

2. The compound according to claim 1, wherein said one or more peptides consist of Ac-AQFHRHKQLIRFLKRA (SEQ ID NO:7).

3. The compound according to any of claims 1-2, wherein said compound is a monomer comprising one of said peptides.

4. A multimeric compound comprising two or more copies of a peptide consisting of the sequence according to SEQ ID NO:1 or comprising two or more copies of a peptide consisting of a variant of the sequence according to SEQ ID NO:1 having a different amino acid at one position, wherein said peptide is capable of inhibiting macrophage activation.

5. An isolated peptide consisting of AQFHRHKQLIRFLKRA (SEQ ID NO:1), or a variant thereof having a different amino acid at one position, wherein said peptide is capable of inhibiting macrophage activation.

6. A pharmaceutical composition comprising at least one compound according to any of claims 1 to 4, or a peptide according to claim 5.

7. A compound according to any of claims 1-4, or a peptide according to claim 5, for use as a medicament.

8. A compound according to any of claims 1-4, or a peptide according to claim 5, for use in the treatment of inflammatory diseases or conditions.

9. The compound or peptide for use according to claim 8, wherein the inflammatory disease or condition is an autoimmune disease or condition.

10. The compound or peptide for use according to claim 8, wherein the inflammatory disease or condition is rheumatoid arthritis.

11. The compound or peptide for use according to claim 8, wherein the inflammatory disease or condition is ischaemic heart disease.

12. The compound or peptide for use according to claim 8, wherein the inflammatory disease or condition is selected from the group consisting of inflammatory diseases of the central nervous system, Alzheimer's disease, Parkinson's disease, Huntington's disease, Multiple sclerosis, meningitis and encephalitis.

13. The compound or peptide for use according to any of claims 8 to 12, wherein the compound is formulated for subcutaneous, intravenous, oral, nasal, pulmonal or topical administration, or parenteral administration supplemented with intraarticular administration into or near joint capsules.

14. Use of a compound according to any of claims 1-4, or a peptide according to claim 5, for the manufacture of a medicament for treatment of inflammatory diseases or conditions.

Patentansprüche

1. Verbindung, bestehend aus einem oder mehreren Peptiden, wobei jedes Peptid aus AQFHRHKQLIRFLKRA (SEQ ID NO:1) oder einer Variante davon mit einer anderen Aminosäure an einer Position besteht, wobei das Peptid in der Lage ist, Makrophagenaktivierung zu hemmen.

2. Verbindung nach Anspruch 1, wobei das eine oder die mehreren Peptide aus Ac-AQFHRHKQLIRFLKRA (SEQ ID NO:7) bestehen.

3. Verbindung nach einem der Ansprüche 1-2, wobei es sich bei der Verbindung um ein Monomer, das eines der Peptide umfasst, handelt.

4. Multimere Verbindung, umfassend zwei oder mehr Kopien eines Peptides, bestehend aus der Sequenz gemäß SEQ ID NO:1, oder umfassend zwei oder mehr Kopien eines Peptides, bestehend aus einer Variante der Sequenz gemäß SEQ ID NO:1 mit einer anderen Aminosäure an einer Position, wobei das Peptid in der Lage ist, Makrophagenaktivierung zu hemmen.

5. Isoliertes Peptid, bestehend aus AQFHRHKQLIRFLKRA (SEQ ID NO:1) oder einer Variante davon mit einer anderen Aminosäure an einer Position, wobei das Peptid in der Lage ist, Makrophagenaktivierung zu hemmen.

6. Pharmazeutische Zusammensetzung, umfassend mindestens eine Verbindung nach einem der Ansprüche 1 bis 4 oder ein Peptid nach Anspruch 5.

7. Verbindung nach einem der Ansprüche 1-4 oder Peptid nach Anspruch 5 zur Verwendung als Arzneimittel.

8. Verbindung nach einem der Ansprüche 1-4 oder Peptid nach Anspruch 5 zur Verwendung bei der Behandlung entzündlicher Erkrankungen oder Zustände.

9. Verbindung oder Peptid zur Verwendung nach Anspruch 8, wobei es sich bei der entzündlichen Erkrankung oder dem entzündlichen Zustand um eine Autoimmunerkrankung oder einen Autoimmunzustand handelt.

10. Verbindung oder Peptid zur Verwendung nach Anspruch 8, wobei es sich bei der entzündlichen Erkrankung oder dem entzündlichen Zustand um rheumatoide Arthritis handelt.

11. Verbindung oder Peptid zur Verwendung nach Anspruch 8, wobei es sich bei der entzündlichen Erkrankung oder dem entzündlichen Zustand um ischämische Herzkrankheit handelt.

12. Verbindung oder Peptid zur Verwendung nach Anspruch 8, wobei die entzündliche Erkrankung oder der entzündliche Zustand aus der Gruppe ausgewählt ist, die aus entzündlichen Erkrankungen des zentralen Nervensystems, Alzheimer-Krankheit, Morbus Parkinson, Chorea Huntington, multipler Sklerose, Meningitis und Enzephalitis besteht.

13. Verbindung oder Peptid zur Verwendung nach einem der Ansprüche 8 bis 12, wobei die Verbindung für die subkutane, intravenöse, orale, nasale, pulmonale oder topische Verabreichung oder die parenterale Verabreichung, ergänzt mit einer intraartikulären Verabreichung in oder nahe Gelenkkapseln, formuliert ist.

14. Verwendung einer Verbindung nach einem der Ansprüche 1-4 oder eines Peptides nach Anspruch 5 für die Herstellung eines Arzneimittels zur Behandlung entzündlicher Erkrankungen oder Zustände.

Revendications

1. Composé constitué d'un ou de plusieurs peptides, chaque peptide étant constitué de AQFHRHKQLIRFLKRA (SEQ ID NO : 1), ou de l'un de ses variants comportant un acide aminé différent au niveau d'une position, dans lequel ledit peptide est capable d'inhiber l'activation des macrophages.

2. Composé selon la revendication 1, dans lequel lesdits un ou plusieurs peptides sont constitués de Ac-AQFHRHKQLIRFLKRA (SEQ ID NO : 7).

3. Composé selon l'une quelconque des revendications 1 à 2, dans lequel ledit composé est un monomère comprenant l'un desdits peptides.

4. Composé multimère comprenant deux copies ou plus d'un peptide constitué de la séquence selon SEQ ID NO : 1 ou comprenant deux copies ou plus d'un peptide constitué d'un variant de la séquence selon SEQ ID NO : 1 comportant un acide aminé différent au niveau d'une position, dans lequel ledit peptide est capable d'inhiber l'activation des macrophages.

5. Peptide isolé constitué de AQFHRHKQLIRFLKRA (SEQ ID NO : 1), ou de l'un de ses variants comportant un acide aminé différent au niveau d'une position, dans lequel ledit peptide est capable d'inhiber l'activation des macrophages.

6. Composition pharmaceutique comprenant au moins un composé selon l'une quelconque des revendications 1 à 4, ou un peptide selon la revendication 5.

7. Composé selon l'une quelconque des revendications 1 à 4, ou un peptide selon la revendication 5, pour une utilisation en tant que médicament.

8. Composé selon l'une quelconque des revendications 1 à 4, ou un peptide selon la revendication 5, pour une utilisation dans le traitement de maladies ou de pathologies inflammatoires.

9. Composé ou peptide pour une utilisation selon la revendication 8, dans lequel la maladie ou la pathologie inflammatoire est une maladie ou une pathologie auto-immune.

10. Composé ou peptide pour une utilisation selon la revendication 8, dans lequel la maladie ou la pathologie inflammatoire est la polyarthrite rhumatoïde.

11. Composé ou peptide pour une utilisation selon la revendication 8, dans lequel la maladie ou la pathologie inflammatoire est une cardiopathie ischémique.

12. Composé ou peptide pour une utilisation selon la revendication 8, dans lequel la maladie ou la pathologie inflammatoire est choisie dans le groupe constitué de maladies inflammatoires du système nerveux central, la maladie d'Alzheimer, la maladie de Parkinson, la maladie de Huntington, la sclérose en plaques, la méningite et l'encéphalite.

13. Composé ou peptide pour une utilisation selon l'une quelconque des revendications 8 à 12, dans lequel le composé

est formulé pour une administration sous-cutanée, intraveineuse, orale, nasale, pulmonaire ou topique, ou une administration parentérale complémentée par une administration intra-articulaire dans ou près des capsules articulaires.

14. Utilisation d'un composé selon l'une quelconque des revendications 1 à 4, ou d'un peptide selon la revendication 5, pour la fabrication d'un médicament destiné au traitement de maladies ou de pathologies inflammatoires.

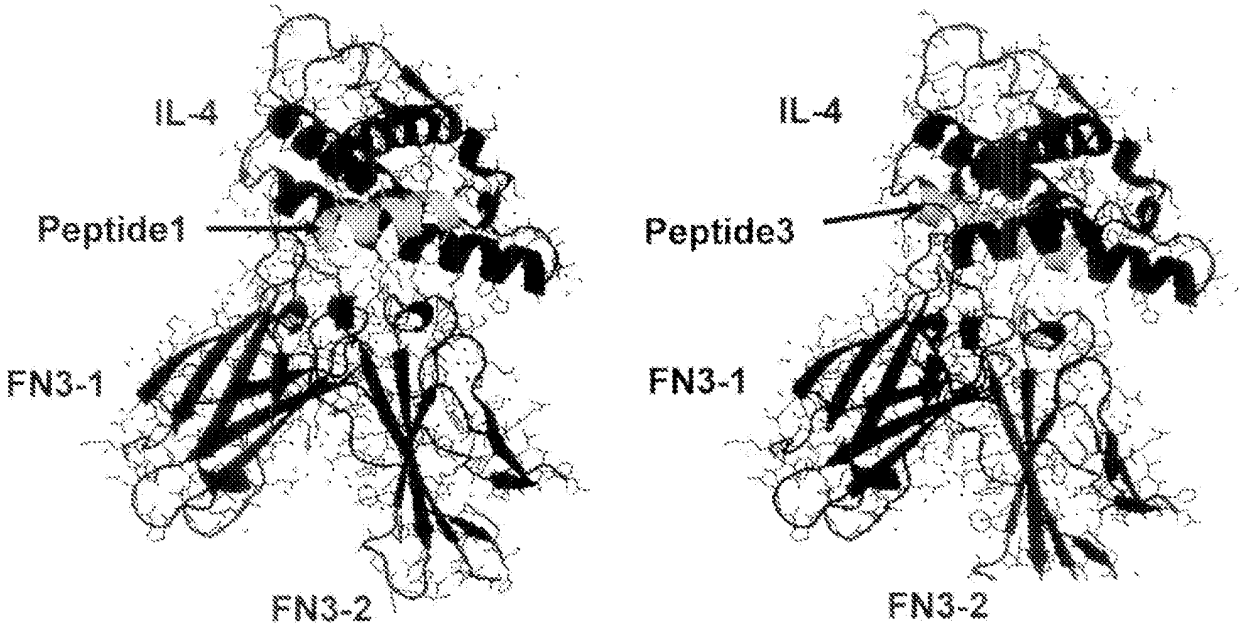

Figure 1

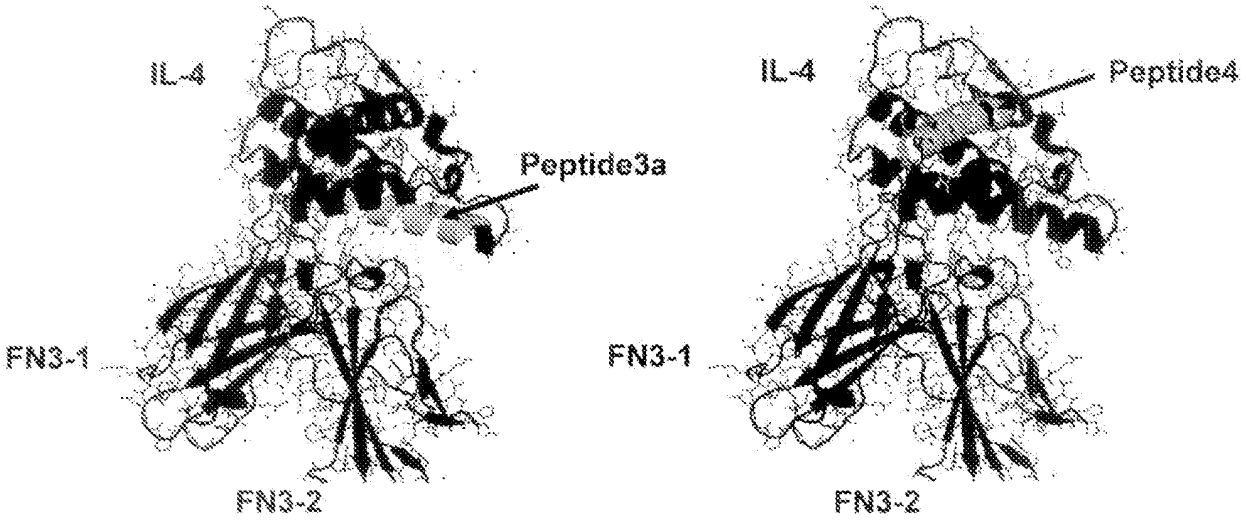

Figure 2

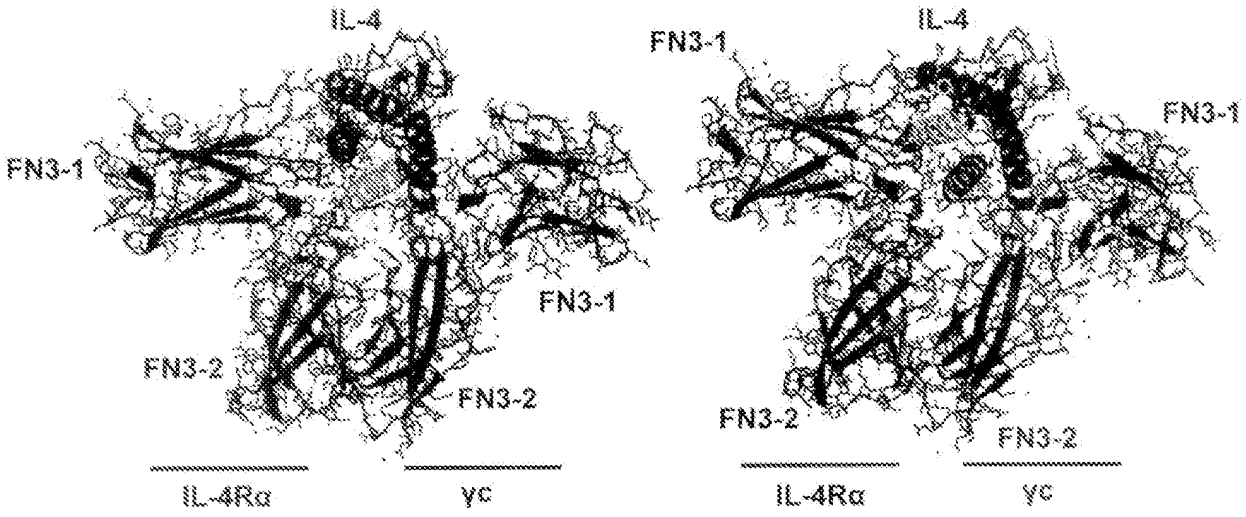
IL-4
FN3-1
FN3-1
FN3-2
FN3-2
IL-4Rα
γc
IL-4
FN3-1
FN3-1
FN3-2
FN3-2
IL-4Rα
γc

Figure 3

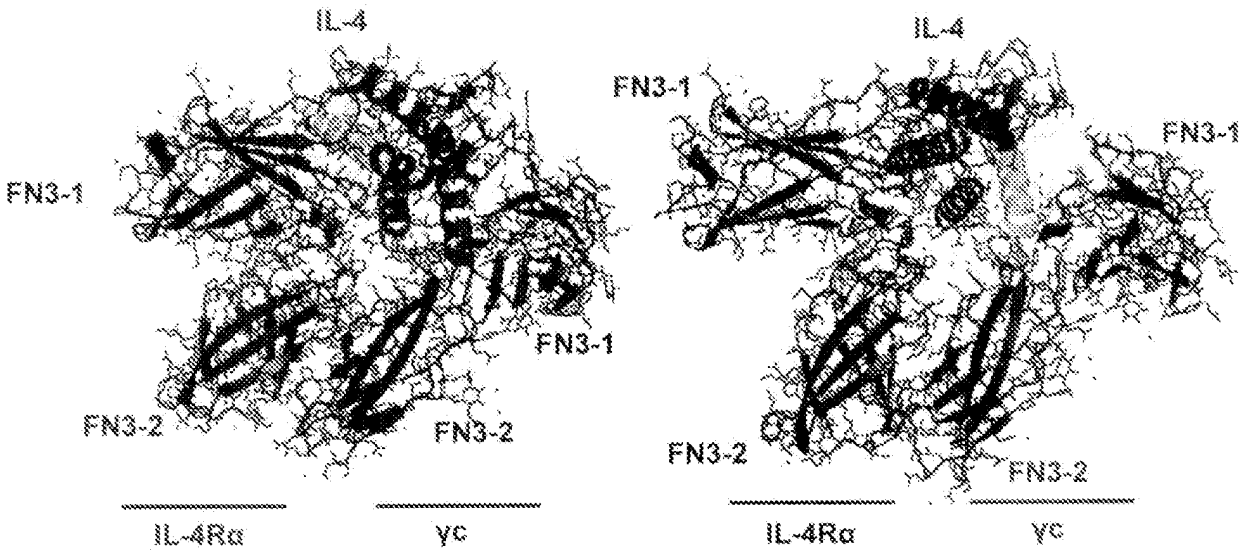
IL-4
FN3-1
FN3-1
FN3-2
FN3-2
IL-4Rα
γc
IL-4
FN3-1
FN3-1
FN3-2
FN3-2
IL-4Rα
γc

Figure 4

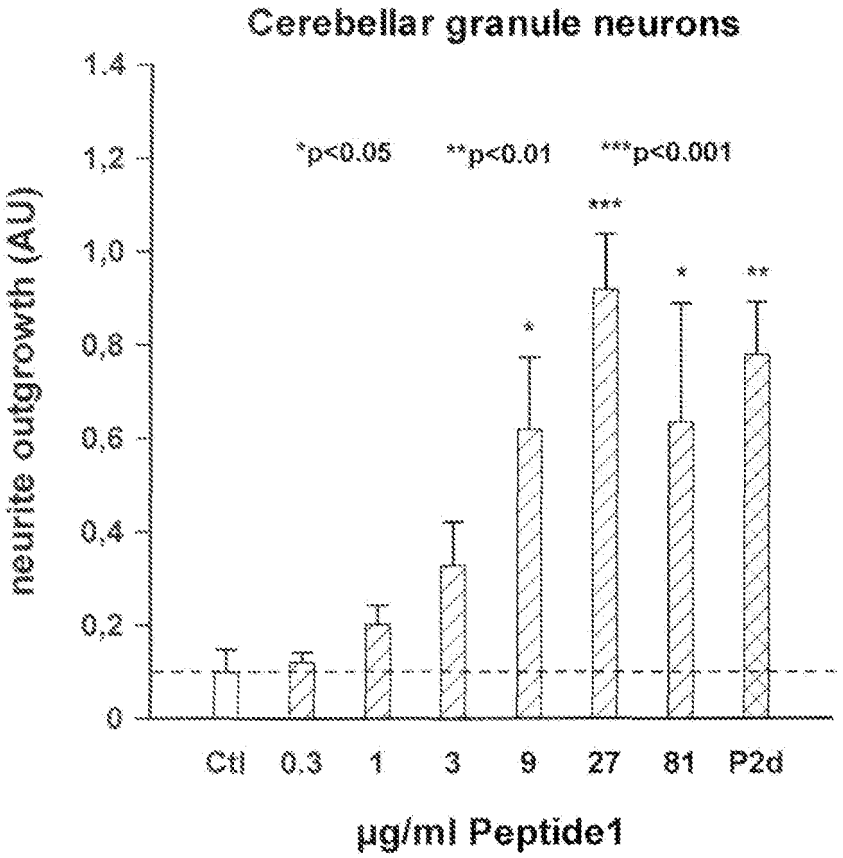
Cerebellar granule neurons
*p<0.05
**p<0.01
***p<0.001
neurite outgrowth (AU)
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0
Ctl
0.3
1
3
9
27
81
P2d
µg/ml Peptide1

Figure 5

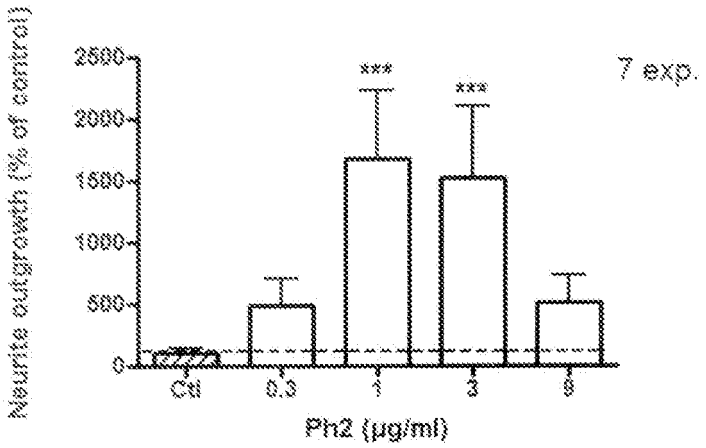
Neurite outgrowth (% of control)
2500
2000
1500
1000
500
0
***
***
7 exp.
Ctl
0.3
1
3
9
Ph2 (µg/ml)

Figure 6

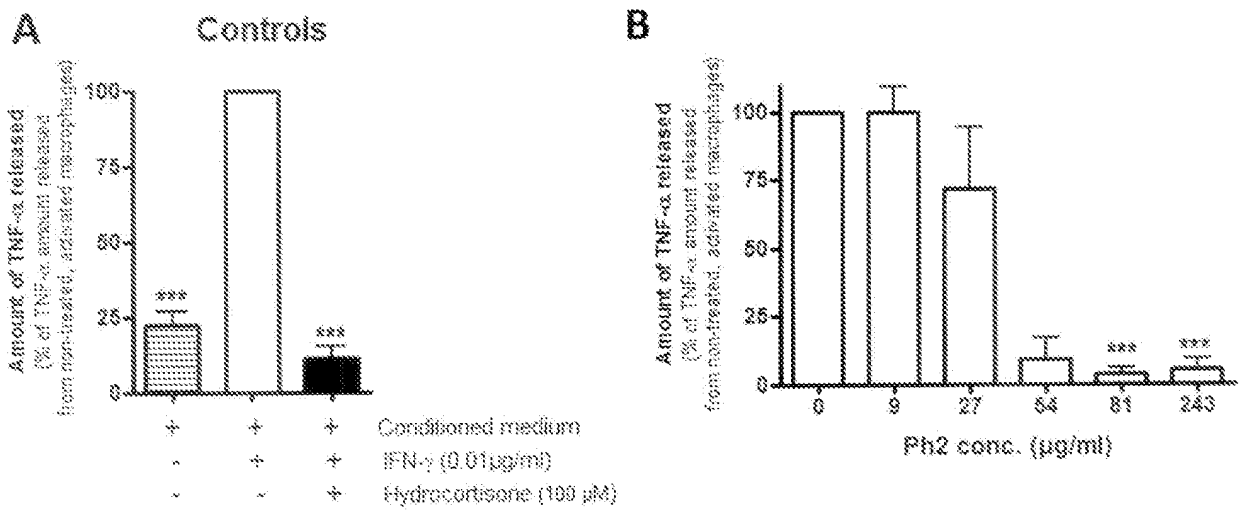
A
Controls
Amount of TNF-α released
(% of TNF-α amount released
from non-treated, activated macrophages)
100
75
50
25
0
***
***
+ + + Conditioned medium
- + + IFN-γ (0.01µg/ml)
- - + Hydrocortisone (100 µM)
B
Amount of TNF-α released
(% of TNF-α amount released
from non-treated, activated macrophages)
100
75
50
25
0
***
***
0
9
27
54
81
243
Ph2 conc. (µg/ml)

Figure 7

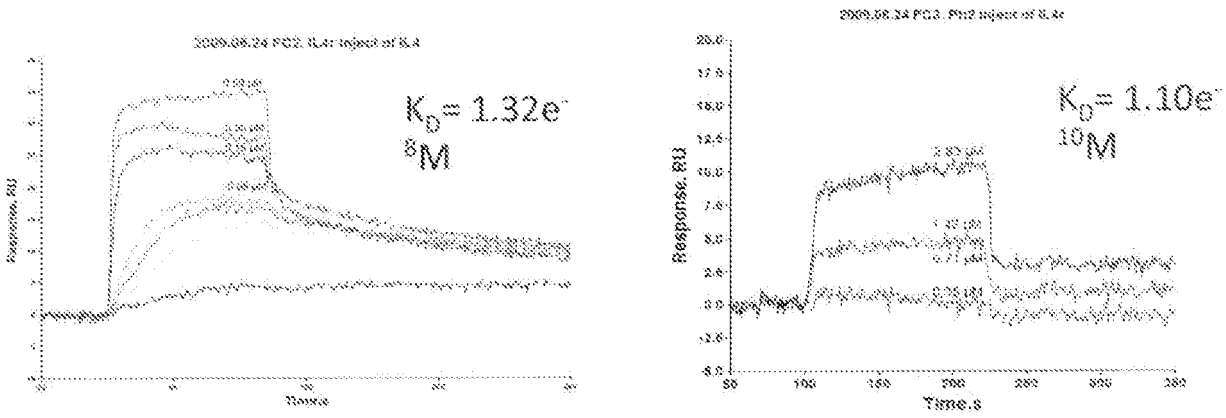

Figure 8

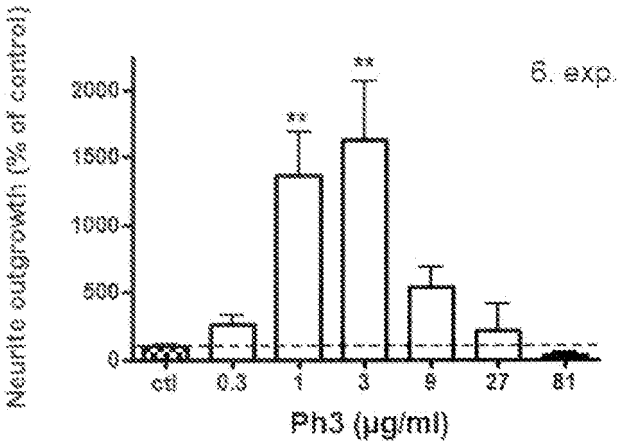
Neurite outgrowth (% of control)
2000
1500
1000
500
0
**
**
6. exp.
ctl
0.3
1
3
9
27
81
Ph3 (µg/ml)

Figure 9

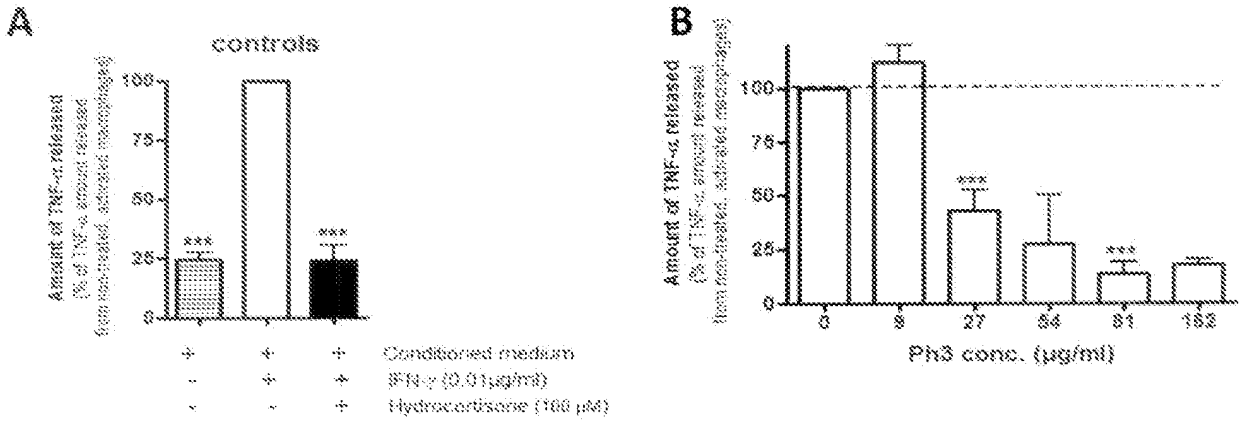
A
controls
Amount of TNF-α released
(% of TNF-α amount released
from non-treated, adjusted macrophages)
100
75
50
25
0
***
***
+ + + Conditioned medium
- + + IFN-γ (0.01μg/ml)
- - + Hydrocortisone (100 μM)
B
Amount of TNF-α released
(% of TNF-α amount released
from non-treated, adjusted macrophages)
100
75
50
25
0
***
***
0
9
27
84
91
183
PH3 conc. (μg/ml)

Figure 10

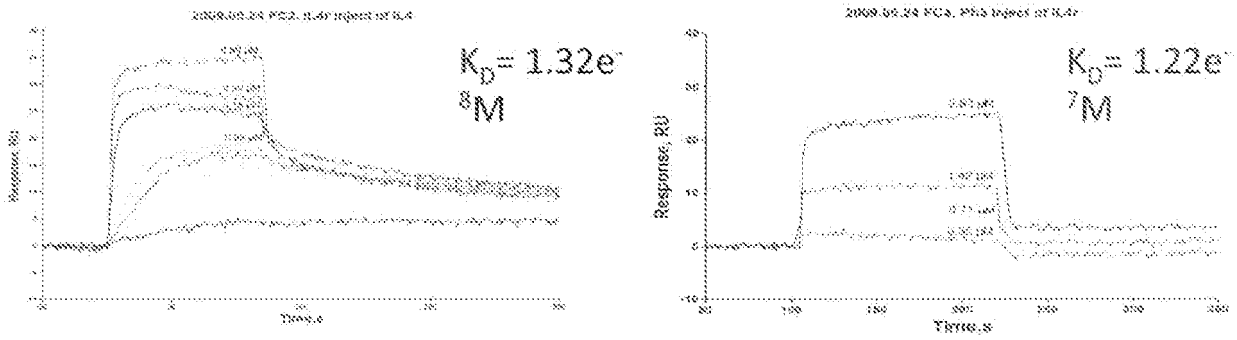

Figure 11

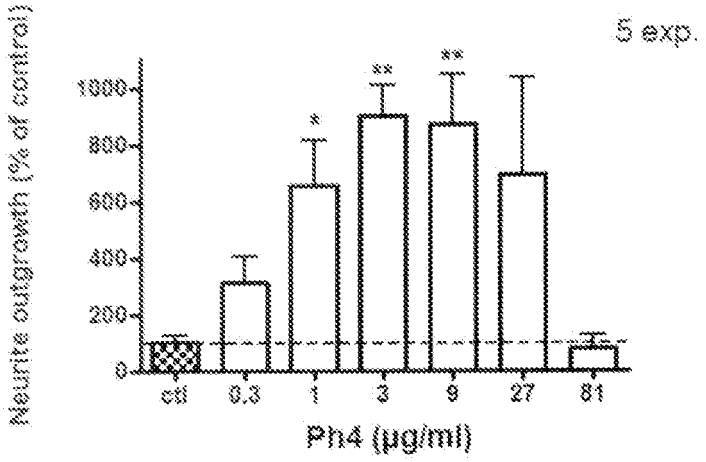
5 exp.
Neurite outgrowth (% of control)
1000
800
600
400
200
0
ctl
0.3
1
3
9
27
81
*
**
**
Ph4 (µg/ml)

Figure 12

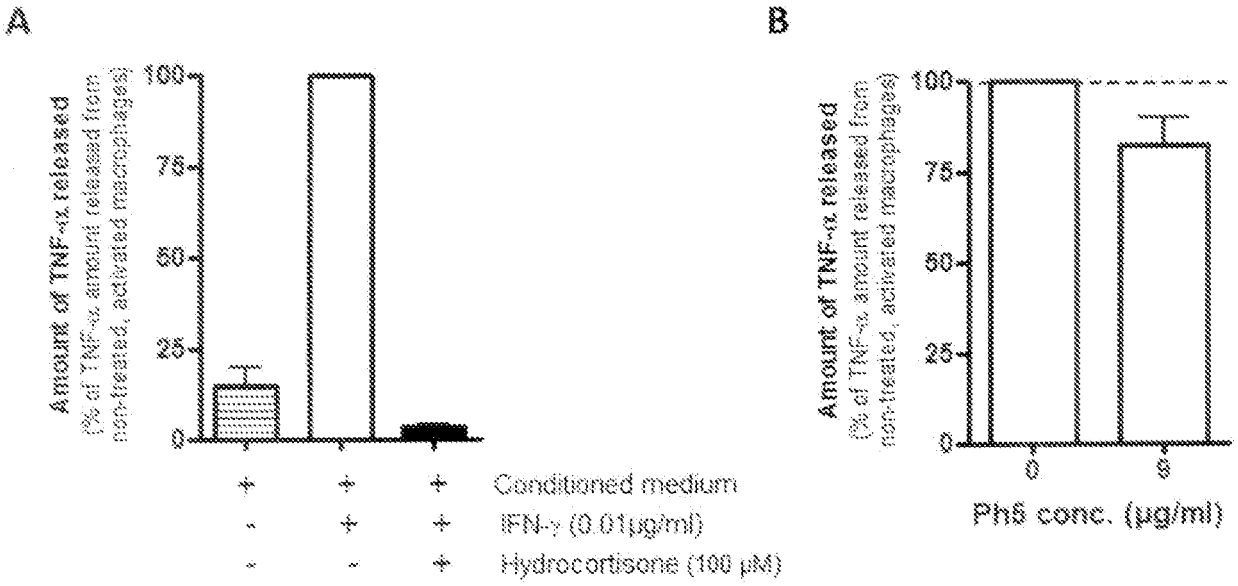
A
Amount of TNF-α released
(% of TNF-α amount released from non-treated, activated macrophages)
100
75
50
25
0
+ + + Conditioned medium
- + + IFN-γ (0.01μg/ml)
- - + Hydrocortisone (100 μM)
B
Amount of TNF-α released
(% of TNF-α amount released from non-treated, activated macrophages)
100
75
50
25
0
0
6
Ph6 conc. (μg/ml)

Figure 13

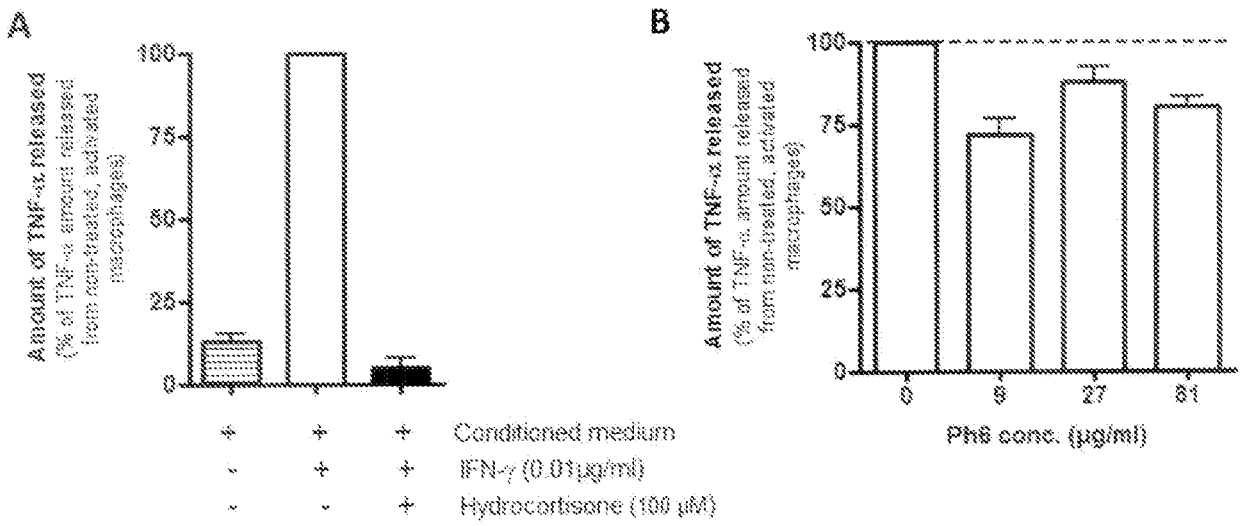
A
Amount of TNF-α released
(% of TNF-α amount released
from non-treated, activated
macrophages)
100
75
50
25
0
+ + + Conditioned medium
- + + IFN-γ (0.01μg/ml)
- - + Hydrocortisone (100 μM)
B
Amount of TNF-α released
(% of TNF-α amount released
from non-treated, activated
macrophages)
100
75
50
25
0
0
9
27
81
PhB conc. (μg/ml)

Figure 14

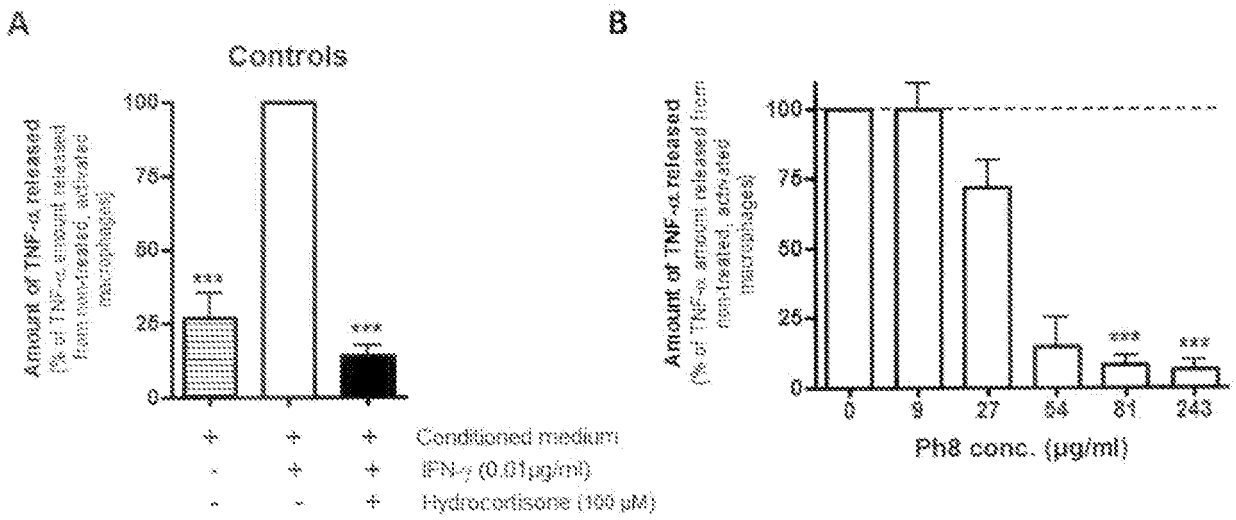
A
Controls
Amount of TNF-α released
100
75
50
25
0
***
***
+ + + Conditioned medium
- + + IFN-γ (0.01µg/ml)
- - + Hydrocortisone (100 µM)
B
Amount of TNF-α released
100
75
50
25
0
0
9
27
84
81
243
***
***
Ph8 conc. (µg/ml)

Figure 15

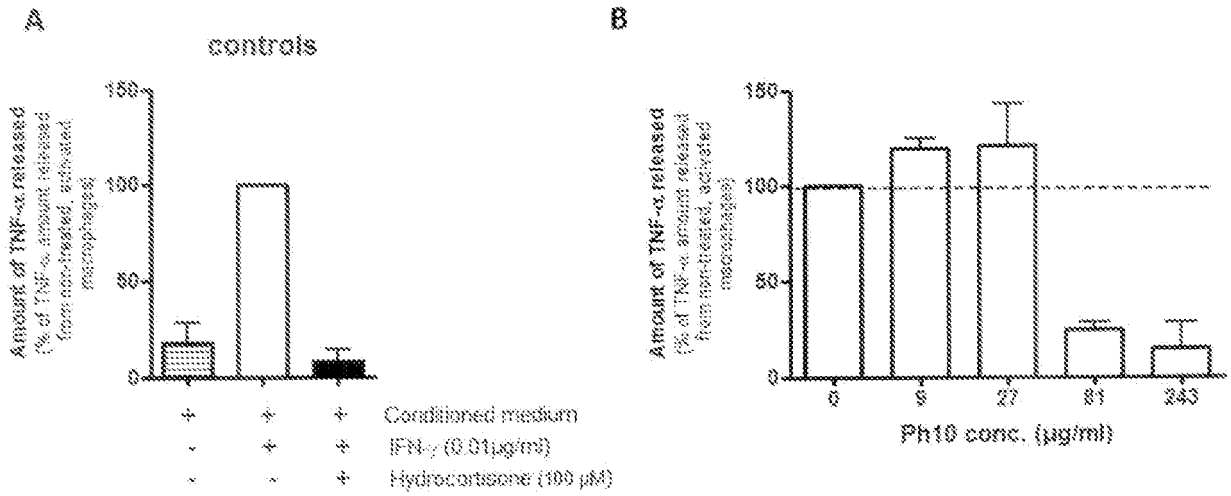
A
controls
150
100
50
0
+ + + Conditioned medium
- + + IFN-γ (0.01µg/ml)
- - + Hydrocortisone (100 µM)
B
150
100
50
0
0
9
27
81
243
Ph10 conc. (µg/ml)

Figure 16

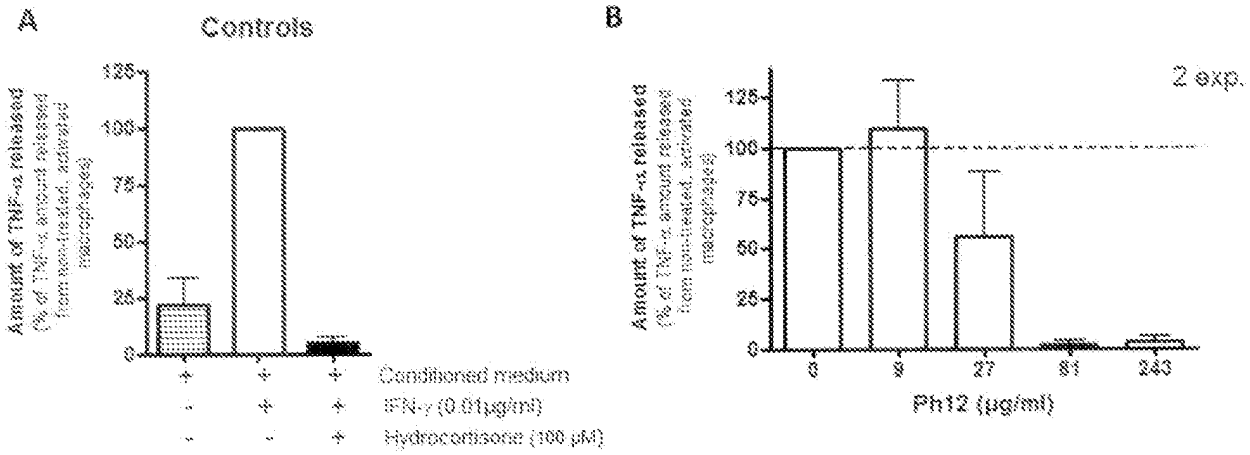
A
Controls
125
100
75
50
25
0
+ + + Conditioned medium
- + + IFN-γ (0.01µg/ml)
- - + Hydrocortisone (100 µM)
B
2 exp.
125
100
75
50
25
0
0
9
27
81
243
Ph12 (µg/ml)

Figure 17

REFERENCES CITED IN THE DESCRIPTION

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